Variation in DISC1 affects hippocampal structure and function and increases risk for schizophrenia

Joseph H. Callicott*, Richard E. Straub, Lukas Pezawas, Michael F. Egan, Venkata S. Mattay, Ahmad R. Hariri, Beth A. Verchinski, Andreas Meyer-Lindenberg, Rishi Balkissoon, Bhaskar Kolachana, Terry E. Goldberg, and Daniel R. Weinberger†

Genes, Cognition, and Psychosis Program, Clinical Brain Disorders Branch, Division of Intramural Research, National Institute of Mental Health, National Institutes of Health, and Department of Health and Human Services, Bethesda, MD 20892

Edited by Edward M. Scolnick, Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA, and approved April 13, 2005 (received for review January 20, 2005)

Disrupted-in-schizophrenia 1 (DISC1) is a promising schizophrenia candidate gene expressed predominantly within the hippocampus. We typed 12 single-nucleotide polymorphisms (SNPs) that covered the DISC1 gene. A three-SNP haplotype [hCV219779 (C)–rs821597 (G)–rs821616 (A)] spanning 83 kb of the gene was associated with schizophrenia in a family-based sample ($P = 0.002$). A common nonconservative SNP (Ser704Cys) (rs821616) within this haplotype was associated with schizophrenia ($P = 0.004$). Based on primary expression of DISC1 in hippocampus, we hypothesized that allelic variation at Ser704Cys would have a measurable impact on hippocampal structure and function as assayed via specific hippocampus-related intermediate phenotypes. In addition to overtransmission in schizophrenia, the Ser allele was associated with altered hippocampal structure and function in healthy subjects, including reduced hippocampal gray matter volume and altered engagement of the hippocampus during several cognitive tasks assayed with functional magnetic resonance imaging. These convergent data suggest that allelic variation within DISC1, either at Ser704Cys or haplotypes monitored by it, increases the risk for schizophrenia and that the mechanism of this effect involves structural and functional alterations in the hippocampal formation.

association | hippocampus | morphometry

Schizophrenia is a common neuropsychiatric disorder with a prominent genetic basis. As suggested by postmortem, animal lesion, and neuroimaging studies, schizophrenia is thought to arise at least in part from neurodevelopmental abnormalities of the hippocampal formation (HF) (1). *In vivo* studies have shown small but significant reductions in HF volume (2) and proton magnetic resonance spectroscopy measures of the intraneuronal marker *N*-acetylaspartate (NAA) (3). Also suggestive of HF dysfunction in schizophrenia, patients have abnormal evoked potentials (4), abnormal performance on episodic memory tests (5), and reduced HF functional MRI (fMRI) activation during episodic memory (6).

According to the prevailing view, schizophrenia is highly heritable, and multiple genetic components are at work (7). Linkage studies have demonstrated several potential candidate loci, including 1q42.1 (8). A balanced translocation within this region (1q42.1;11q14.3) in a large Scottish family was associated with prominent psychopathology (9) and led to the identification of two novel genes, disrupted-in-schizophrenia (DISC) 1 and 2, disrupted by the break (10). Subsequently, three of four studies support an association between allelic variation in the DISC1 gene and risk for schizophrenia (11–14). In the two strongest associations, Hennah and colleagues (12) found a two marker haplotype spanning intron 1 to exon 2 that was undertransmitted in families to female Finnish probands, and Hodgkinson *et al.* (14) found a significant association between schizoaffective disorder and a nonsynonymous SNP in exon 9 (Leu607Phe) in European American cases. Reminiscent of the Finnish study, the latter group also found significantly decreased frequency in schizoaffective subjects of a haploblock wholly encompassed by the two marker haplotype found in the Finnish. In sum, there is support for a relationship between DISC1 and schizophrenia, but these statistical associations are unconvincing on their own.

Given that genetic associations in complex disorders like schizophrenia are typically weak and difficult to replicate, we have taken the approach that convergent data about the effect of variation in the gene on biologic characteristics of the illness that are in turn plausibly related to the biology of the gene can greatly strengthen such statistical results (15, 16). In the present study, we have explored the relationship of variation in DISC1 to biological phenomena that could be predicted *a priori* to be impacted by DISC1 and that are related to the neurobiology of schizophrenia.

DISC1 is a moderately conserved gene that codes for a 93.6-kDa protein of 854 aa with a globular N terminus domain, a coiled C terminus domain, and several coiled-coil domains (17). The functional role of DISC1 is largely unknown, but these distinct domains allow DISC1 protein to interact with both centrosomal and cytoskeletal proteins like NUDEL as well as with membrane associated and signal transduction proteins such as ATF4 and ATF5 (18). There is some evidence that DISC1 is critical during HF development, and it has been proposed as a molecular marker for developing hippocampal neurons (19). The regional expression of DISC1 in brain tissue appears to be conserved within mammals with highest expression in the hippocampus for mouse, rat, and primate (20–22). In early mouse development, DISC1 shows preferential expression in neuronal proliferative zones in HF (e.g., the dentate gyrus) and in limbic regions of cortex (e.g., the anterior cingulate) (19). Truncated DISC1 protein (based on the translocation breakpoint) failed to localize normally (18) and to interact with NUDEL, resulting in reduced neurite outgrowth (23).

Although much remains to be investigated for DISC1, its expression, function, and developmental timing within the hippocampus give the statistical association between DISC1 and schizophrenia a biologic appeal. In this study, we examined the effects of DISC1 genotype on risk for schizophrenia using a family-based association method. We also explored the functional impact of DISC1 variation on phenotypes linked to HF. We focused on (*i*) HF structure using MRI voxel-based morphometry (VBM); (*ii*) HF function via NAA measures; (*iii*) the physiological response of HF during memory tasks as measured by blood oxygenation level-

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Abbreviations: BOLD, blood oxygenation level-dependent; fMRI, functional MRI; HC, healthy comparison subjects; HF, hippocampal formation; LD, linkage disequilibrium; NAA, *N*-acetylaspartate; NIMHGI, National Institute of Mental Health Genetics Initiative; ROI, region of interest; SCZ, patients with schizophrenia; VBM, voxel-based morphometry.

*To whom correspondence may be addressed at: Building 10, Center Drive, Room 4C-216, MSC 1364, Bethesda, MD 20892-1364. E-mail: callicottj@mail.nih.gov.

†To whom correspondence may be addressed at: Building 10, Center Drive, Room 4S-235, MSC 1379, Bethesda, MD 20892-1379. E-mail: weinberd@mail.nih.gov.

Table 1. Demographics

Diagnosis	*N*	Age (±SD)	Gender	Education (±SD)	SES (±SD)	IQ (±SD)	WRAT (±SD)
SCZ	252	36.1 (9.5)	55/252 F	14.0 (2.3)	22.6 (10.6)	92.8 (12.4)	102.9 (12.0)
SIBS	311	37.4 (9.4)	183/311 F	15.7 (2.5)	45.8 (15.5)	106.7 (10.9)	106.4 (11.0)
HC	238	35.2 (10.2)	117/238 F	16.7 (2.9)	47.6 (16.4)	108.1 (8.7)	108.1 (9.7)
Total	801	36.4 (9.7)	355/801 F	15.5 (2.8)	39.0 (18.2)	102.8 (12.8)	105.8 (11.1)

Basic demographics are presented for probands with schizophrenia (SCZ), their unaffected siblings (SIBS), and unrelated healthy comparison subjects (HC). SES, subject's socioeconomic status; IQ, Wechsler Adult Intelligence Scale, revised edition; WRAT, Wide Range Achievement Test.

dependent (BOLD) fMRI; and (*iv*) cognitive performance referable to HF.

We found that a common nonconservative SNP (rs821616, a serine to cysteine substitution at codon 704) and haplotypes containing this SNP were associated with schizophrenia in our sample. This SNP resides within exon 11, which is a site of alternative splicing in human DISC1 (17). Our intermediate phenotype analyses showed that the Ser allele overtransmitted to schizophrenic offspring is associated with several alterations in the human HF in normal individuals and that these alterations resemble findings in schizophrenia. Our data support the idea that DISC1 increases the risk for psychosis and that the mechanism of this effect may involve development and plasticity of the HF.

Methods

Subjects. The Clinical Brain Disorders Branch (CBDB) Sibling Study is an ongoing examination of neurobiological abnormalities related to genetic risk for schizophrenia and all subjects [probands with schizophrenia spectrum disorders (SCZ), their unaffected siblings, and unrelated healthy comparison subjects (HC)] were recruited as described in detail in ref. 24 (see *Supporting Methods*, which is published as supporting information on the PNAS web site). All participants were between 18 and 60 years of age with a premorbid IQ above 70 and gave written informed consent. Only Caucasian subjects of self-reported European ancestry were included herein to avoid stratification and to reduce heterogeneity. DNA genotyping was available for 1,169 subjects, including 252 SCZ, 311 of their unaffected siblings, 238 HC, and 368 parents of SCZ. Table 1 lists the basic demographic characteristics of this sample. Intermediate phenotype measures were obtained for subgroups as described below. We obtained a limited replication sample of parents and SCZ offspring for analysis of family-based association with schizophrenia from the National Institute of Mental Health Genetics Initiative (NIMHGI) (25). The sample we tested included African American ($n = 51$ families) and Caucasian ($n = 67$) with one or two siblings (average 2.06 and 1.87, respectively) with a diagnosis of schizophrenia or schizoaffective disorder, depressed subtype, and available parents. No other phenotypic measures were available for this sample.

DNA Collection and Statistical Analysis. We used standard methods to extract DNA from white blood cells with the Puregene DNA purification kit (Gentra Systems). DISC1 genotyping was performed by using the TaqMan 5′-exonuclease allelic discrimination assay (26). We analyzed these three independent cohorts (European Americans from the CBDB Sibling Study, African Americans from the NIMHGI, and European Americans from the NIMHGI) in two ways (as family based and as case control) for 12 SNPs and "sliding window" two and three marker haplotypes (see Fig. 3, which is published as supporting information on the PNAS web site). Family-based analyses were performed with TRANSMIT (27). Genotype accuracy was assessed by regenotyping within a subsample, and reproducibility was routinely >0.99. We eliminated probable genotyping errors via nonmendelizing transmissions with TRANSMIT and haplotype inconsistency errors via MERLIN (28). All remaining genotypes did not deviate from Hardy–Weinberg equilibrium. Given the number of unaffected siblings available, we additionally performed a combined transmission disequilibrium test/sibling-transmission disequilibrium test (TDT/S-TDT) (29). We measured linkage disequilibrium (LD) between markers as indexed by the D' and delta^2 statistics from parental haplotypes by use of the program LDMAX within the GOLD software package (30).

Intermediate Phenotypes. To minimize the impact of type I errors given the large number of intermediate phenotype measures potentially available, we selectively analyzed the Ser704Cys polymorphism (which was the principal positive SNP via TDT results) against a small number of intermediate phenotypes (24). Because a Bonferroni correction seemed overly stringent given our explicit prior hypotheses of hippocampal dysfunction and the prior probability that DISC1 impacts on hippocampal development, we report uncorrected P values (unless otherwise noted). Nonetheless, multiple testing is of limited concern in our results because only 12 intermediate phenotypes were analyzed relative to Ser704Cys genotype, including neuroimaging phenotypes that already underwent correction for multiple comparisons within image space. As additional protection against multiple sampling errors, the imaging analyses were performed with independent samples.

Availability of the MRI scanner was the only factor determining whether we collected MRI, ^{1}H-MRSI (magnetic resonance spectroscopic imaging), and fMRI data (Table 2). VBM and fMRI analyses have been performed to date only on the normal control samples, because sufficient numbers of high-quality scans to perform genetic analyses have not been available in the other samples. For VBM, ^{1}H-MRSI, and N-back cohorts, Ser704Cys genotype groups did not differ significantly in basic demographic characteristics. For the encoding/retrieval fMRI cohort, Cys carriers tended to have fewer years of education but had the same IQ and higher Wide Range Achievement Test scores, suggesting that both groups were equated for intellectual capacity.

HF Structure. We processed 1.5-T MRI scans for VBM analyses blind to genotype and according to the optimized methodology described by Good and colleagues in ref. 31 and detailed in ref. 32. We examined global effects of genotype on gray matter volume using multiple regression. We controlled for potential confounds in our statistical model for gender, total brain volume, and age (33). We used a hypothesis-driven region of interest (ROI) approach centered on the HF with a significance level of $P < 0.05$ [corrected for multiple voxel comparisons within the HF ROI as selected by using the Wake Forest University PICKATLAS SPM2 toolbox (WFU_PICKATLAS) (34)].

HF NAA Measures. ^{1}H-MRSI was performed on a 1.5-T GE-SIGNA scanner (GE Medical Systems) (3). Metabolite signals are reported as ratios of the area under the peaks NAA/creatine, NAA/choline, and choline/creatine+phosphocreatine as described in ref. 3. Two raters drew the HF ROI blindly with reference to standard anatomical atlases on coplanar structural MRI scans (described in

Table 2. Demographics: MRI intermediate phenotypes and SNP10 (Ser704Cys)

Intermediate phenotype	SNP10	*N*	Age (±SD)	Gender	Education (±SD)	SES (±SD)	IQ (±SD)	WRAT (±SD)
VBM	Ser/Ser	86	35.1 (10.3)	49/86 F	17.2 (3.3)	48.8 (17.1)	107.7 (8.0)	107.8 (10.1)
	Ser/Cys	61	33.8 (9.4)	27/61 F	16.7 (2.3)	49.3 (14.8)	109.1 (9.3)	107.2 (9.5)
	Cys/Cys	10	33.9 (12.2)	3/10 F	17.0 (2.3)	47.7 (18.6)	107.8 (8.5)	112 (7.1)
1H-MRSI	Ser/Ser	109	37.4 (9.4)	46/109 F	15.3 (2.5)	35.3 (17.4)	103.2 (13.5)	106.1 (10.3)
	Ser/Cys	111	34.7 (8.7)	47/111 F	15.2 (2.5)	35.6 (17.9)	102.6 (12.7)	105.5 (10.1)
	Cys/Cys	16	35.1 (8.8)	6/16 F	15.4 (2.6)	33.7 (18.6)	99.7 (12.0)	105.5 (15.2)
N-back	Ser/Ser	18	35.6 (9.5)	6/18 F	17.7 (3.8)	54.3 (16.9)	107.9 (6.8)	104.3 (16.2)
	Cys carriers	24	37.1 (9.0)	14/24 F	17.5 (2.4)	48.6 (18.6)	109.2 (8.2)	106.6 (9.3)
Enc/Ret	Ser/Ser	12	27.7 (5.3)	4/12 F	17.4 (3.0)*	51.1 (16.1)	105.6 (9.2)	103.4 (10.7)**
	Cys carriers	16	26.4 (7.3)	6/16 F	15.9 (1.5)	52.1 (11.2)	107.7 (9.9)	111.2 (5.8)

Samples for MRI phenotypes are presented. Of note, VBM, N-back, and incidental encoding/retrieval (Enc/Ret) analyses were performed only for healthy comparison subjects. SES, subject's socioeconomic status; IQ, Wechsler Adult Intelligence Scale, revised edition; WRAT, Wide Range Achievement Test. *, $P = 0.1$; **, $P < 0.05$.

detail in ref. 3). We transferred the HF ROI onto the ^{1}H-MRSI maps, and the average metabolite values were computed for this region and examined for genotype effects using ANOVA.

HF BOLD fMRI Activation. To test the hypothesis that allelic variation in DISC1 influenced HF information processing, we used cognitive tasks assaying working memory and declarative memory in HC (Table 2). We favor analyses of normal subjects in these data sets to avoid potential confounders related to illness. Our strategy in selecting these tasks was to invoke HF activation in different cognitive settings. HC subjects performed both tasks in the same scanning session by using block designs collapsing correct and incorrect trials. For short-term memory, we used the N-back working memory task described in ref. 35, administered as a simple block design alternating between a two-back (2B) working memory condition contrasted with a no-back (0B) control condition. For declarative memory, we used the incidental episodic memory task described in ref. 36. The task involved incidental encoding and subsequent retrieval of novel, complex visual scenes contrasted with a simple crosshair fixation baseline condition. Whole-brain BOLD fMRI data were collected on a 3-T Signa scanner by using a GE-EPI RT pulse sequence (GE Medical Systems) and analyzed in SPM99 as described in refs. 37–39. For our hypothesis-driven focus on HF function, results from a second-stage random-effects ANOVA were masked with a HF ROI mask as described in ref. 37. Reported loci for genotype effects on task-related activation exceed significance levels of at $P < 0.05$ (corrected for multiple voxel comparisons within the 245-voxel ROI) and are reported with reference to the MNI standard space.

HF Cognitive Function. Subjects performed an extensive cognitive test battery (40). To reduce the number of statistical tests, DISC1 effects were examined in measures related to genetic risk for schizophrenia (40) including tests of the following: episodic memory (logical memory I and II from the Wechsler Memory Scale, revised version, and the California Verbal Learning Test), working memory [the WCST (categories and perseverative errors) and the N back (two back, given separately from the fMRI session)], attention [the *D′* measure from the Gordon Continuous Performance Test], and letter fluency. To control for general intelligence, IQ (from the Wechsler Adult Intelligence Scale, revised edition) and reading comprehension (using the Wide Range Achievement Test) (41) were also included. The effect of DISC1 genotype on cognitive measures was assessed by using ANOVA followed by least significant difference (LSD).

Results

Association Studies. We found evidence for an association between allelic variation in DISC1 and schizophrenia in each of our three independent cohorts, with the strongest individual SNP association ($P = 0.004$) in the CBDB sibling study with overtransmission of the Ser allele of SNP10 (Ser704Cys) (Table 3). Because 12 SNPs were tested, this value survived multiple correction. There was weak evidence for an association with SNP8 (C allele, $P = 0.06$) and SNP9 (G allele, $P = 0.07$). The combined TDT/S-TDT analysis echoed these results: The highest z' scores were found for SNP8 (C allele, $z' = 2.85$, one-tailed $P = 0.002$) and SNP10 (Ser allele, $z' = 2.76$, $P = 0.003$) with weaker evidence at SNP9 (see Table 5, which is published as supporting information on the PNAS web site). Also consistent with the individual marker results, the three marker haplotypes containing SNPs 8–10 were strongly associated with schizophrenia: SNP8 (C allele)–SNP9 (G allele)–SNP10 (Ser) ($P <$ 0.002; Table 4). Again, correcting for the 10 haplotypes tested, this

Table 3. TRANSMIT results: SNPs 1–12

SNP	Celera	dbSNP	Location chromosome1	Distance from SNP1	Description	Amino acid substitution	Allele 1, %	*P* value	Overtransmitted allele	No. overtransmitted
1		rs751229	228803544	0	T/C		50	0.30	2	7
2	hCV12001946	rs1572899	228860495	56951	G/C		57	0.52	2	5
3	hCV12001941	rs1934909	228886284	82740	C/T		12	0.20	1	5
4	hCV12001931	rs1538976	228932634	129090	C/G		81	0.39	2	4
5	hCV1650667		228989106	185562	T/C	Phe607Leu	15	0.20	2	7
6	hCV1650706	rs4079841	229027102	223558	C/T		35	0.17	2	8
7		rs999710	229045948	242404	G/A		62	0.29	1	7
8	hCV219779		229096825	293281	C/A		47	0.06	1	13
9	hCV1433188	rs821597	229137269	333725	G/A		63	0.07	1	12
10	hCV1433135	rs821616	229179603	376059	A/T	Ser704Cys	71	*0.004*	1	18
11			229209780	406236	T/C		71	0.29	1	7
12	hCV9626960	rs1411776	229212492	408948	G/A		86	0.50	1	4

Results ($P < 0.05$) indicated by italics.

Table 4. TRANSMIT results: Three marker haplotypes

1	2									
2	1	2								
3	1	2	1							
4		2	1	2						
5			2	2	2					
6				2	2	1				
7					1	1	1			
8						1	1	1		
9							1	1	1	
10								1	1	1
11									1	1
12										1
Global *P* value	0.44	0.59	0.39	0.19	0.18	0.08	0.14	*0.005*	0.08	0.06
P value	0.18	0.23	0.19	*0.04*	*0.01*	0.09	*0.03*	*0.002*	*0.02*	*0.007*
Frequency	0.04	0.08	0.06	0.09	0.34	0.06	0.22	0.31	0.43	0.43
Obs./exp.	23/19	45/41	35/31	49/43	184/168	40/34	132/119	183/164	243/227	248/229

Results ($P < 0.05$) indicated by italics. Obs./exp., observed/expected.

value survived multiple correction ($P < 0.005$). In SCZ from the Sibling Study sample, SNP 10 showed moderately strong LD with SNP9 and weak LD with SNP12 and with SNP1 (see Tables 6 and 7, which are published as supporting information on the PNAS web site). In HC, LD results were similar. SNP 10 was in strong LD with SNP 9. There was little LD found between other markers. In the case-control analysis of the CBDB Sibling Study sample, we found no frequency differences for alleles or genotypes of any individual SNP.

When we tested for genetic association using the smaller family association cohorts from the NIMHGI, we found additional evidence for an association between DISC1 and schizophrenia. Notably, in the African American NIMHGI cohort, SNP3 showed a positive association (T allele, $P = 0.03$, frequency = 0.94, 3 overtransmissions). We found no significant associations for individual SNPs in the small European American cohort, but there was a trend for overtransmission of the same three marker haplotype seen in the CBDB Sibling Study family association data [SNP8 (C allele)–SNP9 (G allele)–SNP10 (Ser) (global $P = 0.19$, $P = 0.06$, frequency = 0.31, five overtransmissions)]. The results from these two samples, albeit weak in their own right, are unlikely to be due to chance alone given the prior probability of an association.

HF Structure. Thus, our strongest evidence for an association between schizophrenia and an individual SNP in DISC1 was with the Ser allele of SNP10, which led us to focus on the relationship of this allele to our intermediate phenotype measures of HF structure and function. We found a significant genotype effect on hippocampal gray matter volume in our VBM analyses of normal subjects. Ser homozygotes had significantly reduced hippocampal gray matter volume bilaterally when compared with Cys homozygotes [local maxima within left HF at (coordinates $x\ y\ z = -30\ -9\ -18$) ($T = 2.68$) and ($-33\ -29\ -7$) ($T = 2.54$), right ($33\ -14\ -16$) ($T = 1.80$); $P < 0.05$, corrected (see Fig. 1*a*)]. Because of the unbalanced genotype groups, we also compared HC Ser homozygotes ($n = 86$) to all HC Cys carriers ($n = 72$). We again found reduced left hippocampal gray matter volume in the homozygote group [left ($-33\ -28\ -7$)] (see Fig. 1*b*). These effects were not related to age or gender. The effect size for these analyses was small (Cohen's d = 0.36). When we reanalyzed the VBM data in a smaller sample that did not overlap with the other neuroimaging cohorts (Ser homozygotes $n = 54$ vs. Cys carriers $n = 34$), despite the loss of power there was a trend for reduced left HF gray matter volume for Ser homozygotes [($-28\ -29\ -10$), $P = 0.1$] and a similar effect size (Cohen's $d = 0.30$).

HF NAA Measures. We then explored the effects of genotype on our measure of neuronal integrity (NAA), predicting that Ser alleles would have lower NAA. There was a trend toward a main effect of Ser704Cys genotype across all groups in the HF [$F(2, 230) = 2.56$, $P = 0.08$ for the left HF, and $F(2, 230) = 2.60$, $P = 0.08$ for right HF]. Post hoc tests revealed that SCZ Ser homozygotes had significantly lower left HF NAA as compared with SCZ Cys carriers (Ser/Ser vs. Ser/Cys, LSD $P < 0.05$).

HF BOLD fMRI Activation. We predicted that the Ser allele would be associated with a disruption of HF function during memory in normal subjects. During the N-back working memory task, healthy Ser homozygotes showed an uncharacteristic increase in bilateral HF activation compared with normal control Cys carriers [right coordinates: ($22\ -30\ -9$), $T = 2.44$, $P < 0.05$ corrected, and left ($-26\ -26\ -9$), $T = 3.49$, $P < 0.05$ corrected] (Fig. 2*a*). This observation is qualitatively similar to data reported in patients with schizophrenia (39) and in their healthy siblings (38). Hippocampal processing during the declarative memory task also produced a relatively altered physiologic response in Ser homozygotes (see Fig. 2*b*). During encoding of neutral scenes, Ser homozygotes showed decreased activation in bilateral HF [right ($22\ -11\ -16$) ($T = 3.47$) and left ($-26\ -19\ -16$) ($T = 2.76$), for both $P < 0.05$ corrected]. During retrieval of neutral scenes, Ser homozygotes again showed HF underactivation [right ($26\ -26\ -7$) ($T = 2.90$) $P < 0.05$ corrected]. Failure of appropriate hippocampal modulation has been reported with other genes adversely impacting on hippocampal function, such as the Met allele of BDNF at codon 66 (42) and the APOE4 allele (43). Ser-704 effects were thus found in two independent samples and were of moderate to very large effect size

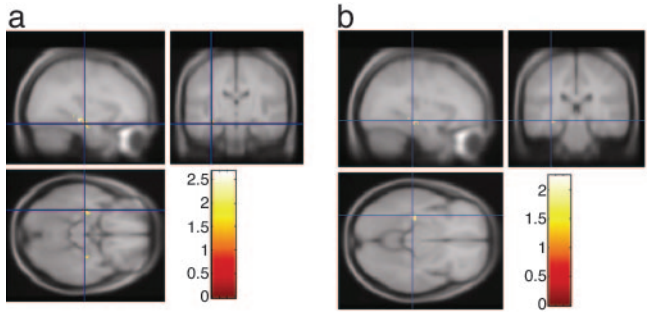

Fig. 1. "Optimized" VBM and SNP10 (Ser704Cys). DISC1 SNP10 deleteriously effects hippocampal size in healthy subjects (HC) ["optimized" VBM analysis within SPM2, $P < 0.05$ corrected (see *Methods*)]. These results were displayed on the standard "MNI305 T1" canonical image within SPM2. (*a*) HC Ser homozygotes ($n = 86$) have reduced gray matter volume compared with HC Cys homozygotes ($n = 10$). (*b*) HC Ser homozygotes also showed reduced gray matter volume compared with HC Cys carriers (Ser/Cys = 62 + Cys/Cys, $n = 10$; total $n = 72$).

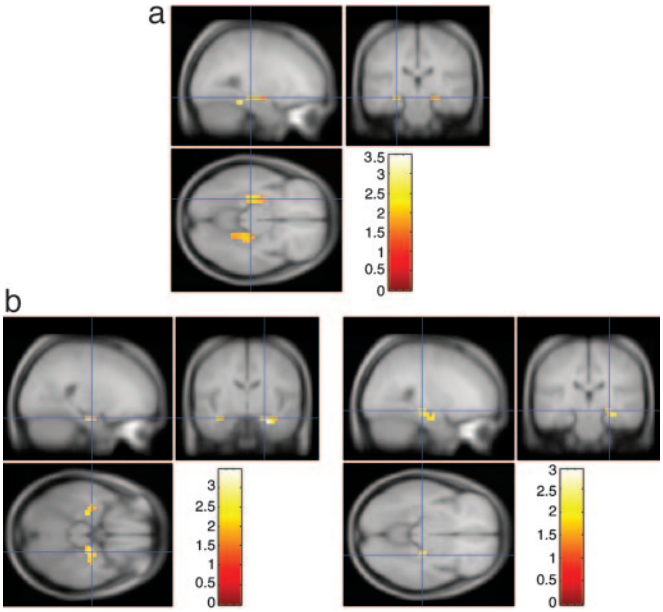

Fig. 2. BOLD fMRI and SNP10 (Ser704Cys). DISC1 SNP10 affects HF activation during both working memory and declarative memory tasks in healthy subjects [SPM99, $P < 0.05$ corrected (see *Methods*)]. (*a*) For the N-back task, results are displayed on the "MNI305T1" canonical image within SPM99. Healthy Ser homozygotes ($n = 18$) showed an apparent atypical increase in HF activation during the N-back working memory task relative to Cys carriers ($n = 24$). (*b*) During the incidental episodic memory task, (encoding on the left and retrieval on the right). Healthy Ser homozygotes ($n = 12$) showed decreased HF engagement during both conditions relative to healthy Cys carriers ($n = 16$).

(Cohen's $d = 0.69–0.79$ for the N-back and $d = 1.01–1.38$ for encoding and retrieval).

HF Cognitive Performance. At the level of observable behavior, HF-related cognition was relatively diminished, although the results were less robust. Homozygous Ser SCZ had reduced performance on the Logical Memory II subsection of the Wechsler Memory Scale (interaction of diagnosis by genotype df = 4, 674; $F = 2.9$, $P = 0.02$), a finding similar to that found in general for schizophrenic probands and their relatives (44). Homozygous Ser allele subjects across all diagnoses had lower WCST category scores [main effect of genotype $F(2, 674) = 3.3$, $P = 0.04$].

Discussion

We have found evidence for an association between variation in DISC1 and schizophrenia in three independent cohorts and converging data from intermediate biologic phenotypes supporting the conclusion that this gene influences hippocampal structure and function in healthy humans. In our largest family-based sample, association within DISC1 was greatest at SNP10, a coding variation (Ser704Cys) with overtransmission of the Ser allele. The most significantly overtransmitted three marker haplotype contained the Ser allele in combination with SNPs 8 and 9. These associations were statistically robust. Moreover, in healthy subjects the Ser allele was associated with structural and functional changes in HF assayed with several biologic measures. In two independent cohorts of healthy volunteers, HF function as measured by BOLD fMRI was relatively abnormal for Ser homozygotes during working and longer term memory challenges. Healthy Ser homozygotes had significantly reduced hippocampal gray matter volume consistent with a structural effect of altered DISC1 expression or function. To a less significant extent, HF NAA levels were reduced, primarily in the left HF of at least SCZ Ser homozygotes, consistent with neuronal impairment. Finally, there were minor cognitive abnormalities including impaired WCST and logical memory across all diagnostic groups. Whereas the NAA and cognitive findings would not survive multiple comparisons, the VBM and fMRI data provide statistically robust confirmation of a role for DISC1 allelic variation in the human hippocampus.

The observations that Ser704Cys is a nonconservative polymorphism residing within or near an alternative splicing domain in exon 11 makes it a particularly attractive candidate as a functional polymorphism of relevance to the function of this gene. However, we do not have any direct molecular evidence that this SNP is functional and it may be a proxy for the true functional loci in the gene. Also, earlier studies that reported evidence for association to DISC1 were negative for this SNP (11, 12, 14), although these studies may involve genetically heterogeneous populations, and allelic heterogeneity is likely to exist within DISC1. In the African American individuals in a second independent sample, a somewhat distant SNP (SNP3) was overtransmitted. Because LD is generally lower in African American populations, this marker may be closer to a functional variant in the gene, although again, there are likely to be important differences in genetic backgrounds within DISC1 between these samples. There was a trend toward association in a third independent cohort of European Americans, although power is limited given the sample sizes in these latter two cohorts. Clearly, additional population samples need to be tested and many more SNPs in the DISC1 gene should be tested as well.

Because the majority of SNPs were not in strong LD in our sample, we have not yet adequately characterized the haplotype structure, which may be very complex. Therefore, it is possible that other risk SNPs are in LD with the Ser704Cys polymorphism, and that increased risk may arise through the overtransmission of an unidentified haplotype that does not include the Ser allele. Regardless of the eventual complexity of the risk haplotypes, the additional contributions of intermediate biologic phenotype data in this study strongly implicate DISC1 allelic variation in HF structure and function. Furthermore, they suggest that the associated Ser allele or related variant may increase risk for schizophrenia via abnormalities in this structure, which has been so often implicated in the pathogenesis of the illness (1). Although not substituting for independent replication, these convergent biologic data lend validity to the interpretation that Ser704Cys (or another variation monitored by it) is physiologically relevant beyond its statistical association with schizophrenia and schizophrenia-spectrum disorders encompassed in our "broad" categorization.

Although there are limitations in each of the intermediate phenotype associations on their own, they tend to support each other when viewed in the context of a larger clinical literature on these phenotypes in SCZ and in siblings of patients with schizophrenia (1). Reduced HF gray matter volume is certainly consistent with a number of reports over many years in schizophrenia (1), as are findings of reduced levels of HF NAA (3). Moreover, atypical overengagement of the HF during the N-back (when HF should be disengaged) is consistent with findings in patients with schizophrenia and in their healthy siblings (38, 39). The presence of HF underengagement in Ser homozygotes during declarative memory processing (when the HF should be engaged) (36) is further support for a relative change in HF processing even in normal subjects, and again, the directionality of the difference is analogous to that described in patients with schizophrenia. Finally, in each of these biologic associations of DISC1, the schizophrenia risk allele (Ser) associated with the relative abnormality.

The association of Ser704Cys to measures of cognition appears relatively weaker than to VBM or fMRI data. However, selective impairment of logical memory in the setting of hippocampal pathology has been reported (45). One might argue that a cognitive measure like the WCST should be most predictive of changes in prefrontal cortical (PFC) function. However, reductions in hippocampal volume have been related to PFC blood flow during the WCST, and to abnormal WCST performance in patients with

schizophrenia (46). Nevertheless, it is conceivable that DISC1 effects are not restricted to the hippocampus, and that the relationship between DISC1 and cognitive impairments referable to cortical systems outside the HF tasks may be more subtle than in HF (and thus undetectable by our other phenotypes) or may arise as a result of abnormal HF connectivity (47). It is interesting that DISC1 had a larger impact on the MRI measures than on cognition. This finding is consistent with other evidence we have reported that the penetrance of gene effects related to psychiatric disorders is greater at the level of brain information processing than at the level of behavior (37, 48). The Ser allele of SNP10 was associated with diminished logical memory and hippocampal NAA measures mainly in schizophrenic subjects, suggesting the necessity for other factors inherited or experienced by probands to produce measurable effects on these phenotypes. These effects may arise as a common result of epistatic interactions with other risk genes (likely given the complex genetic background of schizophrenia itself) and environmental factors. On the other hand, DISC1 variation may produce mild neuronal impairment (such as efficiency of information processing) but not dramatic cognitive deficits on its own. As noted above, because we have no direct evidence that Ser/Cys is the causative mutation, stronger effects may be found with different SNPs within DISC1. For example, translocation carriers have reduced P300 amplitude (8), and a linkage marker near DISC1 was associated with impaired spatial working memory (49). At this time, we also do not have a reliable way to combine information from multiple risk genes as it may be assumed that DISC1 does not increase susceptibility on its own.

By limiting the number of phenotypes studied and focusing on the HF, we attempted to minimize the confounder due to multiple testing. We believe that this hypothesis-driven approach and the convergence of evidence implicating HF biology substantiate the genetic association with diagnosis and should guide efforts at replication in additional independent samples. Conversely, although we feel the demonstration of a relationship between our neuroimaging phenotypes and SNP10 in healthy subjects lends credence to the importance of this allelic variation *in vivo*, we were not able to examine these phenotypes in SCZ or unaffected siblings where such effects may vary given the presence of other positively or negatively modifying genetic and environmental effects.

In conclusion, we have found evidence that variation in DISC1 is associated with increased risk for schizophrenia. Our intermediate biologic phenotype data suggest a mechanism for this increased risk. Variation in the gene marked with the Ser-704 allele appears to impact disadvantageously on HF structure and HF-associated cognitive functions.

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